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BIOLOGICAL ACTIVITY OF *NERIUM OLEANDER* L. (APOCYNACAE) ESSENTIAL OIL ON 5TH LARVAL STAGE OF *SCHISTOCERCA GREGARIA* (FORSKÅL, 1775) (ORTHOPTERA: ACRIDIDAE)

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ABSTRACT

As part of the search of alternative or complementary methods to chemical control against locusts, we study the effect of *N. Oleander* essential oils obtained by hydro distillation of the leaves on 5th larval stage *S. gragaria* (solitary colony) under laboratory conditions. Larvae were fed with determinate cabbage leaves fragments soaked in essential oil. The results show a consumption rejection, decreased body weight and an insecticidal effect. Treated larvae have stopped to feed from the second day of treatment. A significant rate of loss weight of 38, 42% was observed at the end of tests. Cumulative mortality of the treated larvae occurred in the second day with a rate of 16.67 % to 100% on the 7th day, the lethal time 50 (LT50) calculated after adjustment of the logistic regression was of 3.66 days. These preliminary results provide a basis for refining the research on the mode of action of responsible molecules and their toxicity on beneficial insects.

KEYWORDS: Desert Locust, *Nerium oleander*, Essential Oils, Toxicity, Mortality, LT50

INTRODUCTION

The desert locust, *Schistocerca gregaria* (Forskål, 1775) causes extensive damage on food crops in Africa and western Asia, particularly during years with locust outbreaks. Chemical control against this pest has a big economical impact for the international community and significant damages on human health and on the environment (Lecoq, 2004; FAO, 2007). As a response to this situation, a rational control strategy should be settled (Doumandji et al, 2005). To compensate abusive use of insecticides, several new paths are available, among others: entomopathogenic bacteria and fungi, insect growth regulators (IGRs) as well as repellent and biocide plant extracts (Georges et al, 2006; Doumandji-Mitiche and Doumandji, 2008; Outtar et al, 2011; Bissaad et al, 2012).

Botanicals are a promising source of pest control compounds. Over 2000 species of plants are known to possess some insecticidal activity (Jacobson, 1989). Several botanical families are identified for their locust repellent and biocidal properties, among the most cited *Meliaceae* (*Melia azedarrach* L,), *Rutaceae* (*Cestrum parqui* L'Hérit.), *Compositae (Inula viscosa* L.) and *Zygophylaceae (Peganum harmala* L.). These plants have natural allelochemical substances protecting themselves from pests (Idrissi Hassani and Hermas, 2008). One of the most important plants is neem tree *Azadirachta indica* L. which seeds' oil is now marketed as a biopesticide (Popp et al, 2013).

Very few studies have been conducted on the toxicity of *N.oleander* on insects. The plant is toxic to larvae of *Culex pipiens* (aqueous extract) in Tunisia (Barbouche et al, 2001), Rhizotrogini (hydro alcoholic extract) and *Lymantra disparin* in Algeria (Madaci et al, 2008; Kerris et al,2008) and *S. gregaria* (4th larval stage fed with fresh leaves) in Morocco (Bagari et al, 2013). The toxicity of the oleander can change according to several parameters in particular the place and the conditions of sampling. Indeed numerous studies have confirmed that some species have distinct chemotypic race of populations, often separated geographically (Salgueiro et al 1997; Angioni et al 2004). The aim of the present work was to examine effects of *N. Oleander* essential oil on the food intake, growth and mortality of 5th *larval* stage *S. gregaria*

MATERIALS AND METHODS

Biological Materials

5th *larval* stage of *S. gregaria* (solitary colony) used in this study were reared in wooden cages (60 cm x 60 cm x 70 cm) in the laboratory of zoology (University of Mascara, Algeria) under 33 ± 02 °C, 65-75% relative humidity and a 12:12 h (L:D) photoperiod regime. Insects were mainly fed on fresh cabbage, wheat bran and distilled water. *Nerium Oleander* (oleander) mature leaves were collected during flowering stage in the area of Mascara (Algeria).

The plant spontaneously grows in the Mediterranean region as well as in Asia and Oceania sub-tropical regions. In Algeria it is quite common, above all on alluvial deposits and rocky grounds (Chopra et al, 1971). *N. Oleander* is an extremely toxic plant. Taken orally it may cause mortal poisoning **(**Barbosa et al, 2008; Bakkali et al, 2010). Leaves contain about 1.5% of cardenolides, including 0.1 % of oleandrin (Bruneton, 1999). However, the plant contains substances used in medicine and has pharmacological cardiac, antibacterial and antiparasitic properties **(**Delille, 2007; Ibrahim et al, 2007). In Algeria and Morroco, decoctions of leaves are used externally against skin diseases and hair loss (Baba Aissa, 1999; Bnouha et al, 2002).

Extracting of Essential Oil

100 g of fresh *N. Oleander* leaves were hydro-distilled Na_2SO_4 and stored at 4ºC in the dark in a Clevenger-type for 3 hours. The resulting oil was collected, dried over anhydrous sodium sulfate Na_2SO_4 and stored at 4ºC in the dark (Bruneton, 1999). Extracted essential oil was diluted in a determined quantity of acetone. Further on solution was the purpose of biological tests on desert locust.

Experimental Conditions

5^{th} larval stage were placed individually in 1 liter jars with large opening, covered by gauze and have racks to allow larvae roosting and moulting. Larvae were kept in a room under 30 ± 5°C, and 70 ± 5% relative humidity and a 12:12 h (L:D) photoperiod. Three repetitions are performed, each consisting of 10 larvae. 10 other individuals were used as control. The individuals are fasted for 24 hours, and then weighed immediately before testing. In a jar, we placed only fresh cabbage leaves to determine evapotranspiration which adjusts the amount of food consumed and provides information about the humidity (50 ± 5%).Treated larvae are fed with determined fragments of cabbage leaves, *Brassica oleracea* (Brassicaceae), soaked with a quantity of *N. Oleander* essential oil. Control individuals receive cabbage leaves dipped in solvent (acetone). Fragments of cabbage are set 15 to 20 minutes in open air to evaporate acetone before being presented to larvae.

Choice of cabbage as food plant is due to its exceptional nutritional value and palatability by *S. gregaria* (Ould el Hajj et al, 2004). Food intake, body weight-gain and mortality of individuals are taken every 24 hours. The experiment was handled until the total mortality of the treated larvae.

Statistical Analysis

The data obtained were processed by analysis of variance (ANOVA) and comparisons were made at a probability level of 5%. Cochrane test was used to compare mortality of treated larvae. The determination of LT50 (lethal time) and its confidence limit was calculated in a logistic regression model using XLSTAT-Pro. The LT50 is the time for 50% of the animals to die at a particular exposure (Ramade, 2007).

RESULTS

Effect on Food Intake and Weight

Quantity of cabbage consumed and weight of larvae (treated and control) followed the same trend .The results of the effect of *N.oleander* essential oil on food intake are shown in figure 1. The quantities of cabbage consumed by the treated 5th larval stage were significantly reduced ($F_{(1,\ 97)} = 7.92$, $P < 0.0001$) than those recorded in the controls. Effectively we recorded 0.048 ± 0.031g quantities consumed the 1st day after rejection during treatment .They stop feeding from the second day and lose their body weights, while controls improved their weight 10 days after tests and consume everyday an average 2.26 ± 0.38g of cabbage leaves. The results related to weight change larvae are represented in Figure 2. A significant weight loss was recorded for the larvae treated compared to their initial weight because of the refusal of consumption from the second day of treatment.

Indeed, a loss rate of 38.42 % was noted in larvae fed with cabbage leaves soaked in *N.oleander* essential oil while controls have registered a gain weight of 59.55 % at the end of the experiment .The rate of loss weight and the weight gain was calculated by the following formula:(Initial weight-final weight /Initial weight) x100.

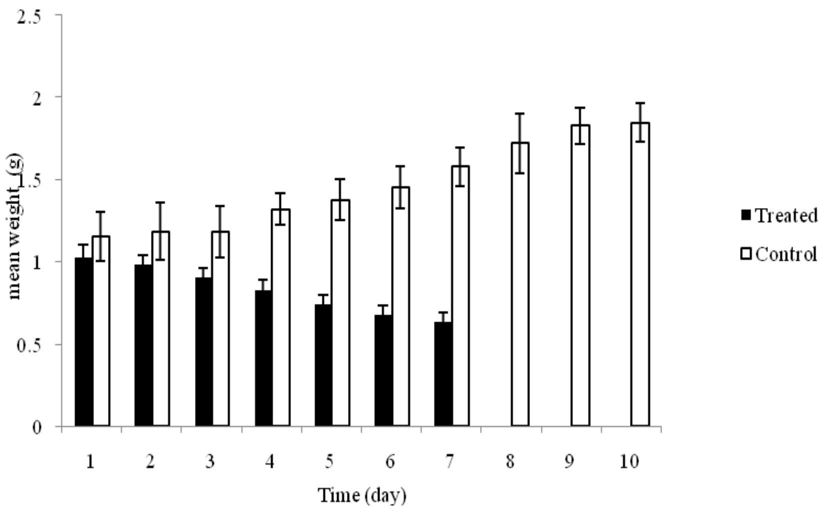

Figure 1: Effect of Nerium Oleander Essential Oil on Mean Weight of 5th Larval Stage *Schistocerca gregaria*

Data Represented the Means ± SD. Experiments was replicated Three Times. Larvae Treated Fed with Cabbage Leave Soaked In Essential Oil

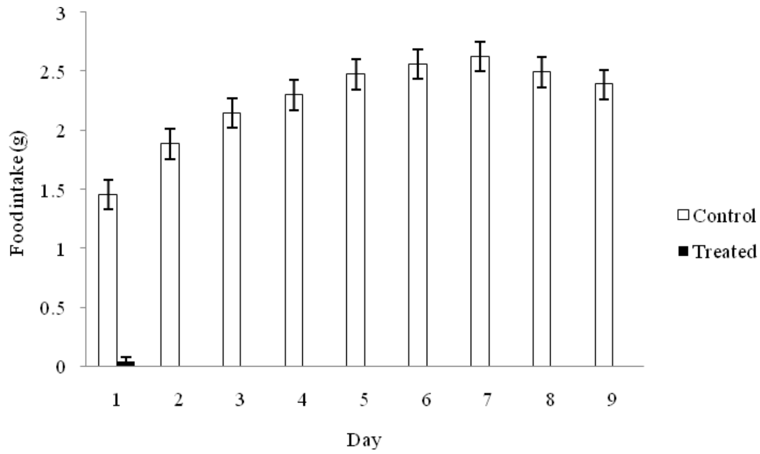

Figure 2: Effect of *Nerium oleander* Essential Oil on Food Intake (G) of 5th Larval Stage *Schistocerca gregaria*

Data Represented the Means ± SD. Experiments Was Replicated Three Times. Quantities of Cabbage Leave Daily Consumed Bylarvae. The Treated Fed with Cabbage Leave Soaked in Essential Oil and Control with Cabbage Soaked in Solvent (Acetone)

Cumulative Mortality and LT50

Larval mortality was daily recorded. The results (figure 3) obtained show that *N.oleander* causes significant mortality among 5th larval stage fed with cabbage soaked in essential oil ($F_{(1.81)} = 53.52$, $p < 0.0001$) compared to control larvae .

Cumulative mortality of the treated larvae occured in the second day with a rate of 16.67 % to 100% on the 7th day , whereas it was of only 10% in controls. the logistic regression model allows calculating the LT50 after adjustment and was obtained by probit transformation of mortality (killed) and logarithm of time (Figure 4).The TL50 value of *N.oleander* essential oil in 5th larval stage *S.gregaria* is 3,66 days who is statistically reliable (Chi 2 = 30.74; p < 0 , 0001).In table 1 are represented the values of lethal time (50,90 ,99 %) and their confidence limits 95% .

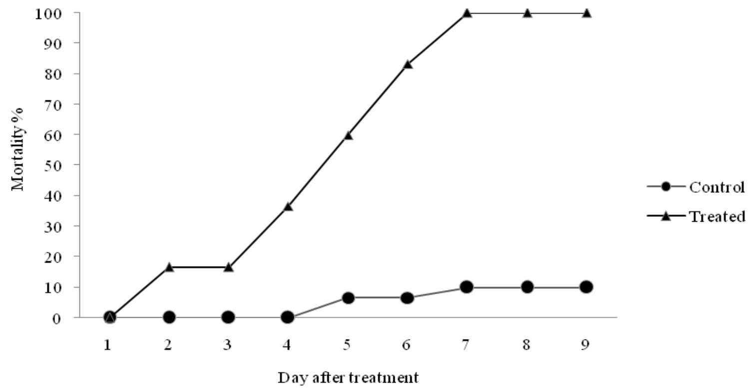

Figure 3: Effect of *Nerium oleander* Essential Oil on Cumulative Mortality rate (%) of 5th Larval Stage Larvae *Schistocerca gregaria.* Experiments were Replicated Three Times. Larvae Treated Fed with Cabbage Leave Soaked in Essential Oil

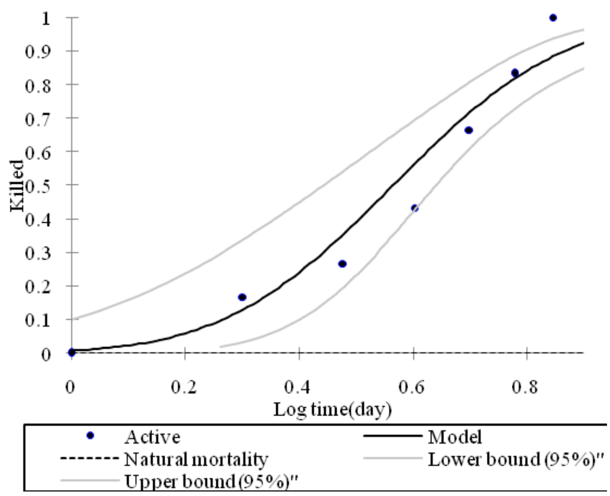

Figure 4: Logistic Regression of 5th Larval Stage *Schistocerca gregaria* Mortality by Log (Time (Day) Lethal Effect of Nerium Oleander Essential Oil. Logistic Regression Obtained by Probit Transformations of the Larvae Mortality (Killed) and Logarithm of Time Using XLSTAT-Pro (P<0.05)

Table 1: .Lethal Time (Day) (50, 90, 99%) of *N. oleander* Essential Oil On 5th Larval Stage *S. gregaria* and its Confidence Limits 95%

Lethal Time (LT)	Value (Time)	Lower Limits 95%	Upper Limits 95%
50	3,66	2,77	4,36
90	7,32	6,19	9,48
99	12,87	9,83	21,67

Lethal values obtained by the probability analysis after adjusting the logistic regression model . Using XLSTAT-Pro(P<0.05).

TL 90 and TL 99 are given as indicative.

DISCUSSIONS

Food intake and body weight gain are indicators of growth for animals (Armbruster and Hutchinson, 2002). *N. oleander* essential oil causes a rejection of consumption, decreased body weight and an insecticidal effect. Several studies indicate a slowdown in growth of 5th larval stage *S. gregaria* subjected to extracts of *Eucalyptus globulus ,Azeradarachta indica*, *Mélia azerdarach*, *Euphorbia guyoniana,inola viscosa*, *Calotropis procerea*), *Glinus litoides* and *Peganum harmala* (Ould ahmadou et al, 2001 ;Ould el hadj et al, 2003 ; Abbassi et al,2004; Abbassi et al, 2005 ; Kemassi et al, 2010). Variability of recorded weight loss rates is related to plants extracts effects on larvae. Indeed Ould el hadj *et al.(* 2003) and Kemassi *et al.(* 2010) report that leave extracts of *A.indica* and *Eucalyptus occidentalis* respectively give weight loss rate of 56 %, and 19.5% of 5th larval stage *S.gegaria.*

The choice of a plant as a food by an insect depends on relative amounts of agents stimulating or inhibiting the absorption of food present in the plant. In the case of locust, food intake is preceded by a behavioral recognition sequence: it explores the surface of the leaf with its maxillary palps before biting. The rejection of the plant usually occurs after the bite (Le Gall, 1989). The *N. oleander* inhibits food intake and has a repellent effect against 5th larval stage larvae *S. gregaria* .The mode of action of essential oils is becoming better known in insects (Ngamo and Hance, 2007), they exert physiological and physical effects. Physiological effects can affect neurotransmitters octopamine which has a regulatory effect on the heart rate, motor and metabolism (Chiasson and Beloin , 2007).

In our study, the insecticidal efficacy of *N. oleander* essential oils led to a total mortality of *S. gregaria* larvae the 7th day after treatment, while Bagari et al. (2013) obtained, on 4th larval stage fed exclusively with fresh leaves of the same plant, a total mortality after 12 days. For the extracts of *Peganum harmala*, *Azadirachta indica* and *Euphorbia guyoniana,* the total mortality occurs at the 16th, 10th and 14th day of treatment (Ould el Hadj et al , 2003; Kemassi et al , 2010). Thus, variability of mortality of larvae noted by these authors is mainly due to the action of the plant (toxic, repellent or anti feedant) and the type of extract used (aqueus extract, essential oil, fresh leaves). The TL 50 we obtain (3,66 days) is lower than those recorded by Aqueous extracts of *A.indica* (7,5days), *M.azedarach* (8.2days), *E. globulus* (10.4 days) and by essential oil of *Cleome arabica* (6,69 days) (Ould el hadj *et al*,2003,Lebbouz,2010). Thus *N.oleander* appears more toxic than all plants mentioned above.*N. oleander* appears more toxic than the majority of plants involved. Cardiac glycosides constituents of *N. oleander* are toxic recognized (Bruneton. J, 2001), the main one being oleandrin (Bakkali et al, 2010), its insecticidal properties deserve further investigation.

CONCLUSIONS

Nerium oleander essential oil has been toxic for 5th larval stage larvae *Schistocerca gregaria*. It causes consumption rejection, decreased body weight and an insecticidal effect. This result provides the basis for further research on the mode of action of responsible molecules and toxicity on auxiliary insects including bees.

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REFERENCES

1. Abbassi, K, Atay Kadiri, Z, & Ghaout S. (2004).Activité biologique des feuilles de *Calotropis procera* (Ait. R. Br) sur le criquet pèlerin (*Schistocerca gregaria*, Forsk. 1775). *Zool. baetica*, 15, 153-166.
2. Abbassi, K, Mergaoui, L, Atay Kadiri Z, Stambouli A. & Ghaout,S. (2005). Effet des extraits de *Peganum harmala* L. (Zygophyllaceae) sur le Criquet pèlerin *Schistocerca gregaria* (Forskål, 1775). *Zool. Baetica* 16, 31-46.
3. Armbruster, P, and Hutchinson, R. A. (2002). Pupal mass and wing length as indicators of fecundity in *Aedes albopictus* and *Aedes geniculatus* (Diptera:Culicidae). *J. Med. Entomol*, 39, 699 -704.
4. Angioni, A., Barra, A., Cereti, E., Barile, D., Coïsson, J.D.,Arlorio, M., Dessi, S.,Coroneo, V., Cabras, P. (2004).Chemical composition, plant genetic differences, antimicrobial and antifungal activity investigation of the essential oil of *Rosmarinus officinalis* L, *J. Agric. Food Chem* , 52, 11, 3530-3535.
5. Baba Aissa, F. (1999). *Encyclopédie des plantes utiles. Flore d'Algérie et du Maghreb*. Libraire Moderne, Rouïba Ed, Alger, Algerie,80 p.
6. Bagari, M, Bouhaimi, A, Ghaout, S, &. Chihrane, J. (2013).The toxic effects of *Nerium oleander* on larvae of the desert locust *Schistocerca gregaria* (Forskål, 1775) (Ortoptera, Acrididae). *Zool. baetica*, 24, 193-203
7. Bakkali, H, Ababou, M, Nassim Sabah, T, Moussaoui ,A, Ennouhi, A., Fouadi,F.Z , Siah, S, Ihrai ,H. (2010).Chemical burns caused by the shrub *Nerium oleander*.*Ann Burns Fire Disasters*, 23(3), 128-130.

8. Barbouche, N, Hajlem, B, Georges, L, & Ammar, M. (2001). Contribution à l'étude de l'activité biologique d'extraits de feuilles de *Cestrum parqui* L'Hérit. (Solanaceae) sur le criquet pèlerin *Schistocerca gregaria* (Forsk.).*Biotechnol. Agron. Soc. Envir*, 5 (2), 85-90.

9. Barbosa, R.R, Fontenele-Neto, J.D, Soto-Blanco, B. (2008).Toxicity in goats caused by oleander (*Nerium oleander*).*Research in Veterinary Science*. 85(2), 279-281.

10. Bissaad, F.Z, Youcef, M, Bounaceur, F, & Doumandji Mitiche, B. (2012).Activité biologique d'un biopesticide le Green muscle sur le tégument du criquet pèlerin Schistocerca gregaria (Forskål, 1775) (Orthoptera,Acrididae).*Nature & Technologie*,06 , 51 -58

11. Bnouha, M.M, Mekhfi, H, Legssyer, A, Ziyyat, A. (2002).Ethno pharmacology forum. Medicinal plants used in the treatment of diabetes in Morocco.*Int. J. diabetes et Metabolism*,10, 33-50.

12. Bruneton, J. (1999). Pharmacognosie. Phytochimie, plantes médicinales. Ed. Tec & Doc, Paris,éd. *médicales internationales*. Cachan, 3e édition, 463-730.

13. Bruneton J, (2001). Plantes toxiques. Végétaux dangereux pour l'Homme et les animaux. Tec & Doc, Paris, éd. *édicales internationales*, Cachan. 2e édition, 129-136.

14. Chiasson, H, & Beloin, N. (2007). Les huiles essentielles, des biopesticides «Nouveau genre».*Antennae*, 14 (1), 3-6.

15. Chopra, L.C, Abrol, B.K, HandaK, L. (1971). *Les plantes médicinales des régions arides considérées surtout du point de vue botanique.* Premiére partie, 45-50.

16. Delille, L. (2007).Les plantes médicinales d'Algérie, Berti éd.. Alger,Algérie. 141-142

17. Doumandji-Mitiche, B, & Doumandji, S. (2008). Quelques agents biologiques susceptibles d'être utilisés en lutte antiacridienne. *Revue des régions arides*, 1154 - 1158

18. Doumandji, S, Doumandji-Mitiche, B, Ould El Hadj, M. D, & Harizia, A. (2005).Place de lutte rationnelle contre la sauterelle pèlerine, *Schistocerca gregaria* (Forskal, 1775) (Orthoptera-Cyrtacanthacridinae). IVème journée nationale d'Acridologie, INA-El Harrach (Alger), 6 Mars 2005, 13-14.

19. FAO. (2007).L'avenir des biopesticides dans la lutte contre le criquet pelerin. Atelier intenationnale, Say, Sénégal, 12-15 Février, 37p.

20. Georges, K, Jayaprakasam, B, Dalavoy, S. S, And Nair, M. G.(2006).Pest-managing activities of plant extracts and anthraquinones from *Cassia nigricans* from Burkina Faso. *Biores. Tecn*, 99, 2037-2045.

21. Ibrahim, K. A, Khalagi, S, Youssef, D, Khan, I, Mesbah, M.(2007).Stimulation of oleander in production by combined *Agrobacterium tumefaciens*mediated transformation and fungal elicitation in *Nerium oleander* cell cultures.*Enzyme and Microbial Technology*, 41, 331-336.

22. Idrissi Hassani, L. M, & Hermas, J. (2008).Effets de l'alimentation en *Peganum harmala* L.(Zygophyllaceae) sur le tube digestif du criquet pèlerin *Schistocerca gregaria* Forsk. (Orthoptera, Acrididae).*Zool. baetica*, 19, 71-84.

23. Jacobson, M. (1989).Botanical pesticides, past, present and future. In “Insecticides of plant origin ”.Amason ,J.T, ed . Proc. Amr. Chem. α Soc. Washington, D. C.:1- 10..
24. Kemassi, A, Boual, Z, Ould El Hadj Khelil, A, Dadi Bouhoun, M, & Ould El Hadj M.D. (2010). Activité biologique de l’extrait d’*Euphorbia guyoniana* (Boiss. & Reut.)(Euphorbiaceae) sur les larves du cinquième stade et sur les adultes de *Schistocerca gregaria* (Forskål, 1775) (Orthoptera-Acrididae).*Annales des Sciences et Technologie*, 2(1) ,61-70.
25. Kemassi, A, Boual, Z, Lebbouz, M. ,Dadi Bouhoun, M, Saker, M.L, Ould Elhadj-Khelil ,A. & Ould El Hadj,M.D .(2012). Etude de l’activité biologique des extraits foliaires de *cleome arabica*l. (capparidaceae).*Lebanese Science Journal*, 13, 81-97.
26. Kerris, T, Djebili, Y, Amoura, S, Bouguerra, S. & Rouibah, M.(2008). Essai d’utilisation du Laurier rose *Nerium oleander* L. en lutte biologique contre le *Lymantria dispar*.2ème Conférence Internationale sur la Biodiversité des Invertébrés en Milieu Agricole et Forestier, INA, Alger: 1-6.
27. Lebbouz, I. (2010). Activités biologiques des extraits foliaires de Cleome arabica L. Capparidaceae) chez *Schistocerca gregaria* (Forskål, 1775). Mémoire de magister. Université de Biskra, Algerie.
28. Lecoq, M.(2004). Vers une solution durable au problème du Criquet pèlerin.*Sécheresse*, 15 (3), 217-224.
29. Le Gall, P. (1989).Le choix des plantes nourricières et la spécialisation trophique chez les Acridoidea (Orthoptères). *Bull. Ecol. Ento.* 20(3) 245-261.
30. Madaci, B, Merghem, R, Doumandji, B, Soltani, N. (2008). Effet du *Nerium oleander*, laurier-rose, (apocynacees) sur le taux des proteines, l’activite de l’ache et les mouvements des vers blancs rhizotrogini, (colepoptera, scarabaeidae).*Sciences & Technologie*, 27,73-78.
31. Ngamo, L.S.T, & Hance, T.H. (2007).Diversité des ravageurs, des denrées et méthodes alternatives de luttes en milieu tropical. *Tropicultura*, 25(4), 215-220.
32. Ould Ahmadou, M. L, Bouaichi, A. & Idirissi Hassani, L. M. (2001). Mise en évidence du pouvoir répulsif et toxique de *Glinus litoides* (Aizoaceae) sur les larves du Criquet pèlerin *Schistocerca gregaria* (Forskål, 1775) (Orthoptera – Acrididea). *Zool. Beatica*, 12,109-117.
33. Ould El Hadj, M. D, Tankari Dan-Badjo, A, Halouane, F. (2003). Etude comparative de la toxicite de trois substances acridifuges sur les larves du cinquieme stade et sur les adultes de *Schistocerca gregaria* forskål, 1775(Orthoptera, Cyrtacanthacridinae). *Courrier du Savoir* , 03, 81-86.
34. Ould El Hadj, M. D, Tankari Dan-Badjo, A, Halouane, F. (2004). Etude du cycle biologique de *Schistocerca gregaria(*forskål, 1775) sur chou (*Brassica oleracea)* en laboratoire, *Courrier du Savoir*, 05 , 17-21.
35. Outtar, F, Doumandji-Mitiche, B, Mouhouche F, & Doumandji, S. (2011).Méthodes alternatives en lutte antiacridienne. Quatrième conférence internationale sur les méthodes alternatives en protection des cultures lille , 8,9 et 10 mars 2011.
36. Popp , J, Petö K, & Nagy, J. (2013). Pesticide productivity and food security. A review. *Agron. Sustainable Dev*, 33, 243-255.

37. Ramade, F. (2007). Introduction à l'écotoxicologie: fondement et application. éd .*Tec et Doc Lavoisier*, Paris,France .618 p.Salgueiro, L.R, Vila, R, Tomi, F, Figueiredo, A.C, Barroso, J.G,Cañigueral, S, Casanova, J, Cunha, A.P, Adzet, T. (1997). Variability of essential oils of Thymus caespititius from Portugal. *Phytochemistry* 45: 307–311.